Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 447–451, January 1996
Genetics

The *Drosophila melanogaster* dodo (*dod*) gene, conserved in humans, is functionally interchangeable with the *ESS1* cell division gene of *Saccharomyces cerevisiae*

(WW domain/peptidylprolyl *cis–trans* isomerase/transgenic organisms/redundancy/degeneracy)

R. MALESZKA*†, S. D. HANES†‡, R. L. HACKETT‡, H. G. DE COUET§, AND GEORGE L. GABOR MIKLOS†¶

*Visual Sciences Group, Research School of Biological Sciences, The Australian National University, GPO Box 475, Canberra ACT 2600, Australia; ‡Wadsworth Center, New York State Department of Health, 120 New Scotland Avenue, Albany, NY 12201-2002; §Department of Zoology, University of Hawaii at Manoa, 2538 The Mall, Honolulu, Hawaii 96822; and ¶The Neurosciences Institute, 10640 John Jay Hopkins Drive, San Diego, CA 92121

Communicated by Melvin Green, University of California, Davis, CA, October 16, 1995

ABSTRACT **We have sequenced the region of DNA adjacent to and including the flightless (*fli*) gene of *Drosophila melanogaster* and molecularly characterized four transcription units within it, which we have named tweety (*twe*), flightless (*fli*), dodo (*dod*), and penguin (*pen*). We have performed deletion and transgenic analysis to determine the consequences of the quadruple gene removal. Only the flightless gene is vital to the organism; the simultaneous absence of the other three allows the overriding majority of individuals to develop to adulthood and to fly normally. These gene deletion results are evaluated in the context of the redundancy and degeneracy inherent in many genetic networks. Our cDNA analyses and data-base searches reveal that the predicted dodo protein has homologs in other eukaryotes and that it is made up of two different domains. The first, designated WW, is involved in protein–protein interactions and is found in functionally diverse proteins including human dystrophin. The second is involved in accelerating protein folding and unfolding and is found in *Escherichia coli* in a new family of peptidylprolyl *cis–trans* isomerases (PPIases; EC 5.2.1.8). In eukaryotes, PPIases occur in the nucleus and the cytoplasm and can form stable associations with transcription factors, receptors, and kinases. Given this particular combination of domains, the dodo protein may well participate in a multisubunit complex involved in the folding and activation of signaling molecules. When we expressed the dodo gene product in *Saccharomyces cerevisiae*, it rescued the lethal phenotype of the *ESS1* cell division gene.**

The increasing number of gene knockouts in *Mus musculus* that have little developmental impact under laboratory conditions may attest to the widespread occurrence of compensatory mechanisms in a mammalian genome (1–4). Similar results are found in other eukaryotes. In *Saccharomyces cerevisiae*, >70% of transcription units, when individually mutated, have no obvious effects (5), while in *Drosophila melanogaster* and *Caenorhabditis elegans*, at least 60% of the mutagenized loci are nonvital ones (6, 7). Such deletion and knockout results are usually evaluated in the context of redundancy (8–10) and less so in the broader frameworks of degeneracy (11, 12) or nonfunctionality of different genomic compartments (5, 6). To determine why some genetic networks are relatively unperturbed by single-gene knockouts whereas others are seriously compromised requires knowledge of the interacting components of each network. The most interesting avenue then is to determine how a given network has evolved in different evolutionary lineages, what consequences it has had for morphological change, and whether the same or similar developmental end points have been reached by differently constituted networks (13–16).

Our long-term goal is to understand the genesis of particular networks in different phyla. To approach this, we have been using as our foundation the vital as well as nonvital genes in a 2-megabase region of *D. melanogaster*; an area subjected to saturation mutagenesis having a large number of chromosomal rearrangements (17). We are (*i*) delineating those transcription units and regulatory regions whose total removal from the genome has only limited consequences for developmental processes, (*ii*) examining those whose deletion or mutation leads instead to cell or organismal lethality; and (*iii*) characterizing homologs of the genes and the regulatory networks in which they are involved from other phyla (17–22).

We have previously isolated and described the *D. melanogaster* flightless (*fli*) gene and its homologs in both *C. elegans* and *Homo sapiens*, and in this paper we report on our isolation of the dodo (*dod*) gene.‖ We have found that the dodo transcription unit is related to the *ESS1* gene of *S. cerevisiae* (23, 24), mutation of which leads to cell death (23). To determine whether the *ESS1* and dodo gene were functionally interchangeable, we expressed the normal fly gene in yeast cells containing defective copies of the *ESS1* gene and examined its effects in terms of cell viability and spore outgrowth; the dodo gene fully rescued the lethality of *ESS1* null mutants.

To ask whether dodo was an essential gene in *D. melanogaster*, we constructed transgenic flies carrying overlapping deficiencies that totally deleted dodo from the genome and examined the viabilities and flight characteristics of these flies. In contrast to the *ESS1* gene in yeast, removal of dodo from the fly genome does not lead to drastic consequences on viability. Loss of dodo function is compensated for by a duplicate copy, by other unrelated proteins, or by protein complexes which perform similar functions. We have examined these results in the general context of redundancy and degeneracy in biological systems.

MATERIALS AND METHODS

Transgenic and Deficiency Analysis. The portion of the flightless landscape used for transgenic analysis is a 10.2-kb *Xho* I genomic fragment ligated into the pW8 vector (21) and injected into *yw*; Δ *2-3 Sb/TM6* embryos. It contains a complete copy of the flightless transcription unit and a portion of the 5′ parts of the tweety (*twe*) and dodo (*dod*) transcription units. This integrant was recombined onto the different chromosomal rearrangements shown in Fig. 1 *Top* by standard

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PPIase, peptidylprolyl *cis–trans* isomerase; TPI, triose phosphate isomerase.

†To whom reprint requests should be addressed.

‖The sequences reported in this paper have been deposited in the GenBank data base (accession number U35140).

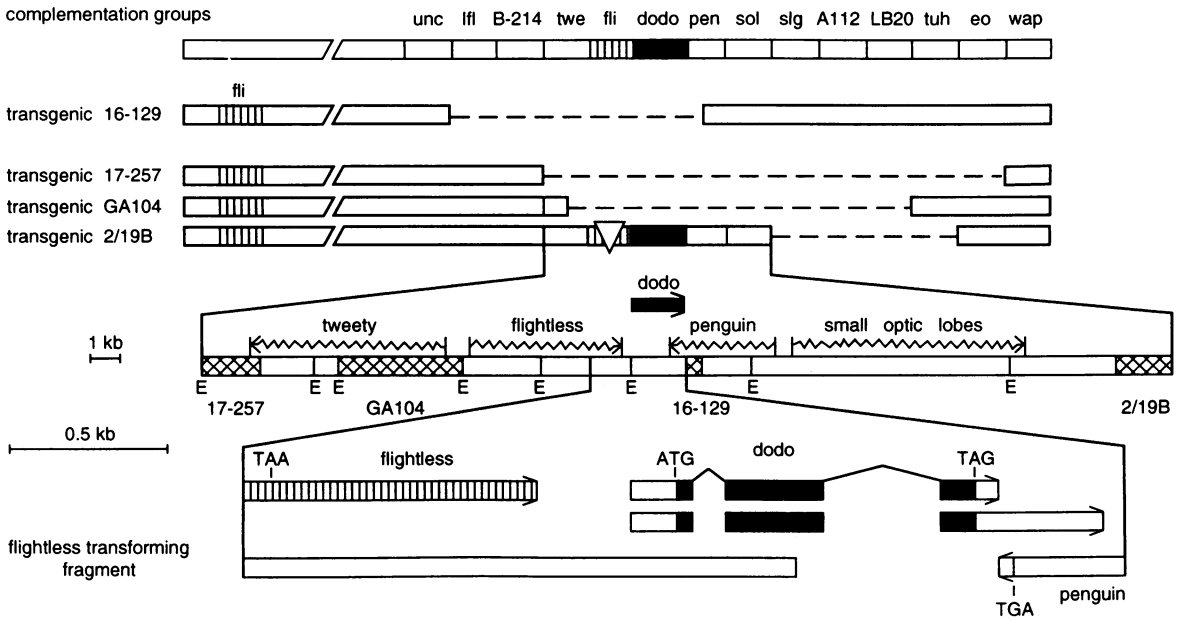

FIG. 1. (*Top*) Genetic and molecular maps of the flightless (*fli*) region: genetic complementation groups and characteristics of the rearranged chromosomes. Chromosome 2/19B carries two mutations, a 5.4-kb insertion in the *fli* transcription unit, which is probably a mobile element, and a deficiency outside of the flightless region. (*Middle*) The five complete transcription units, the *Eco*RI (E) restriction sites, and the extent of the four chromosomal breakpoints (cross-hatched boxes). (*Bottom*) Intron–exon structure of the dodo transcription unit and the positions of the termination codons in the flightless, dodo, and penguin genes. The most proximal extent of the flightless transforming fragment is as shown.

genetic crosses. The viabilities of flies carrying different deficiency combinations were estimated by comparing the number of experimental adults remaining in vials after 8 days relative to their full sib controls. Flies of different genotypes were individually tested for their flight abilities as described (18).

Molecular Characterization of *D. melanogaster* cDNAs. Fourteen *D. melanogaster* cDNAs were isolated from larval and pupal phage λgt10 libraries by using the 3.8-kb genomic fragment containing the dodo (*dod*) and penguin (*pen*) transcription units as a probe (Fig. 1 *Middle*). Ten of these emanated from the dodo gene and four from the adjacent penguin locus. cDNA fragments were subcloned into plasmid and M13mp10 vectors and sequenced as described (19–22). The genomic DNA encompassing the dodo transcription unit was also sequenced, and the cDNAs were aligned on it. Database searches were performed at the National Center for Biotechnology Information (NCBI) by using the BLAST network service, and multiple sequence alignments were generated with CLUSTAL W.

Yeast Expression Constructs. The dodo gene was expressed in yeast cells using the constitutive triose phosphate isomerase (TPI) promoter. The pTPI-dodo constructs (denoted pdodo) were made by insertion of a 1.1-kb *Eco*RI fragment of a full-length dodo cDNA into the *Bam*HI site of pJK305-TPI (a gift from J. Kamens, Harvard University), by using *Eco*RI–*Bam*HI adaptors. These plasmids carry the yeast 2-μm replicator (2μm) and a LEU2 selectable marker. Plasmid YEpHESS carries the yeast *ESS1* gene (23).

Yeast Complementation Assays. Yeast strain MGG3/pSH-U (*Mat***a**/*MATα ura3/ura3 leu2/leu2 his3/his3 ess1*::*URA3/ESS1*) or a homozygous disruption derivative of it (*ess*1::*URA3/ess1*:::*URA3*) that carries *ESS1* on an episomal plasmid (YEpHESS, 2μm, *HIS3*) were used (23). For curing experiments, a diploid disruption strain (*ess1*::*URA3/ess1*::*URA3*) carrying an *ESS1*-containing plasmid was transformed with the appropriate pdodo. It was serially passaged (1:50 dilution) in liquid complete synthetic medium lacking leucine for 6 days in the absence of selection for the *ESS1*-containing plasmid, but with selection for the pTPI-dodo plasmids (2μm, *LEU2*). Cells were plated, and phenotypes of individual colonies were scored by replica plating to appropriate selective media. For tetrad analysis, the heterozygous disruption strain (*ess1*::*URA3/ESS1*) was transformed with different dodo cDNA-containing plasmids. Cells were induced to undergo sporulation on 1% potassium acetate plates, tetrads were dissected, and haploid segregants were grown on rich medium. Growth was scored after 3 days, with segregation of the chromosomal *URA3* and of plasmid-borne *LEU2* and *HIS3* markers being scored by replica plating to appropriate media.

RESULTS AND DISCUSSION

Genomic Manipulations and Biological Consequences. The genetic complementation groups and the known transcription units in the region of at least 300 kb from uncoordinated (*unc*) to extra organs (*eo*) are shown in Fig. 1 *Top* together with the four deficiency chromosomes. The positions and known limits of the four rearrangement breakpoints that we determined (22) and the transcription units that we uncovered in this smaller 30-kb region are illustrated in Fig. 1 *Middle*. The dodo transcription unit, its overlap with the 3′ end of the penguin transcription unit, its intron–exon structure, and its coding and noncoding regions are shown in the 3-kb region of Fig. 1 *Bottom*.

We knew from our previous studies that it was not possible to recover viable adults simultaneously deficient or interrupted in the tweety, flightless, dodo, and penguin genes unless a transgenic copy of the normal flightless gene was present elsewhere in the genome (18, 21). As we had previously generated only a single transgenic copy which could not be recovered as a homozygote, we performed further transformations to recover a transformant on the X chromosome. This allowed us to avoid dosage compensation problems and to facilitate construction of stocks. Having obtained such an integrant, the same wild-type copy of this flightless transcription unit was then recombined onto each of the deficiency-bearing chromosomes to yield *fli*$^+$ *16-129/fli*$^+$ *17-257; fli*$^+$ *16-129/fli*$^+$ *GA104* and *fli*$^+$ *16-129/fli*$^+$ *2/19B* individuals, which developed to adulthood. We then compared the viability and flight abilities of flies carrying two different overlapping deficiency combinations—namely, *fli*$^+$ *16-129/fli*$^+$ *17-257*, and

fli$^+$ *16-129/fli*$^+$ *GA104*—with individuals in which only the flightless transcription unit had been inactivated—namely, *fli*$^+$ *16-129/fli*$^+$ *2/19B*. These latter have only a single normal copy of each of the tweety, dodo, and penguin genes (Table 1).

The two different deficiency combinations yield almost identical results; flies deficient or interrupted in the tweety, dodo, and penguin transcription units are 79% and 76% as viable as their full sib controls and 94% and 93% of them, respectively, have normal flight abilities (Table 1). We do not as yet know the individual contribution that absence or inactivation of each of the transcription units makes to this decrease in viability; even finer transgenic analysis of each of them and their associated regulatory elements is required to determine this. However, it is clear that the tweety, the dodo, and the penguin products are not essential for development to adulthood, nor are they required for the integrated neuromuscular activities needed for flight control. These data are congruent with saturation mutagenesis studies in which the flightless gene was found to be the only vital locus in this part of the chromosome (17).

The dodo (*dod*) Locus. We determined the sequences of three full-length dodo cDNAs together with the 5′ and 3′ ends of a number of smaller cDNAs. The underlying genomic DNA was also totally sequenced (G.L.G.M., H.G.d.C., and R.M., unpublished data). We uncovered two classes of dodo transcripts that differ in the lengths of their 3′ untranslated regions (Fig. 1 *Bottom*), and the gene has two introns, the first of which is not always removed. The putative 5′ end of the dodo transcription unit is 279 bp from the 3′ end of the flightless transcript, whereas the 3′ ends of dodo and penguin overlap by 353 bp. In other respects, such as intron size, the dodo transcription unit *per se* is unremarkable.

The Predicted dodo Protein and Its Relatives. Our data base searches revealed that the predicted 166 amino acid dodo protein showed 44% identity at the amino acid level with the ESS1 and PTF1 proteins of *S. cerevisiae* (23, 24). It became clear after minor sequence corrections that *ESS1* and *PTF1* are the same gene, which is single copy in the yeast genome. Furthermore, we found that dodo protein has excellent sequence similarity to human-expressed sequence tags deposited in data bases and that it has a peptidylprolyl *cis–trans* isomerase (PPIase) domain in common with a number of prokaryotic and eukaryotic PPIases (24–28). The yeast, fly, and human dodo sequences are aligned in Fig. 2 together with a newly described bacterial protein termed "parvulin," which has the highest sequence similarity to the PPIase domains.

The WW Domain. The ESS1, dodo, and human predicted proteins consist of two domains. The first is the WW domain of ≈40 amino acids (Fig. 2), which is found in a number of unrelated proteins involved in cell signaling or regulation—e.g., dystrophin and utrophin, involved in Duchenne and Becker muscular dystrophies; and mouse Nedd4, implicated in embryonic development and nervous system function (29). The WW domain consists of β-strands arranged around four conserved aromatic residues, which together with a hydrophobic core and various charged residues is indicative of well-characterized domains involved in protein–protein interactions (29). Indeed, it has very recently been shown that the WW domain of the Yes-associated protein, YAP, binds to identical proline-rich motifs found in two newly identified proteins (30).

Table 1. Viabilities and flight abilities of individuals carrying different combinations of chromosomal rearrangements relative to their full sib controls

Genotype	Viability	Individuals able to fly normally, %
fli$^+$ *16-129/ fli*$^+$ *2/19B*	1.00	98
fli$^+$ *16-129/ fli*$^+$ *17-257*	0.79	94
fli$^+$ *16-129/ fli*$^+$ *GA104*	0.76	93

The controls in each case are *fli*$^+$ deficiency/*FM7* females. The numbers of individuals scored in the viability experiments were 1474, 1791, and 1892, respectively. The numbers of flies individually tested for flight were 107, 105, and 138, respectively. In the controls, 383 *fli*$^+$ deficiency/*FM7* full sib females were tested; all flew normally.

The PPIase Domain. As is clear from Fig. 2, the bulk of the dodo protein sequence has excellent sequence similarity to a new family of PPIases (EC.5.2.1.8), enzymes involved in protein folding and unfolding that catalyze the *cis–trans* isomerization of Xaa-Pro peptide linkages. The prototypical member of this family is the parvulin protein of *Escherichia coli* (27), which is the smallest of the known PPIases. Its close relatives are lipoprotein PrsA from *Bacillus subtilis*, SurA from *E. coli*, protease maturation protein PrtM from *Lactococcus lactus*, and the NifM family from nitrogen-assimilating bacteria *Azotobacter* and *Klebsiella pneumoniae*; all are now thought to function in protein folding (28).

The three motifs that are thought to be diagnostic for PPIases (28) are seen in Fig. 2. Parvulin shares motifs I, II, and III with the eukaryotic sequences but lacks the WW domain. It has been suggested for PPIases that (*i*) motifs II and III contain a flexible loop with a conserved glycine and that these loops are involved in binding a peptide substrate, and (*ii*) the conservation of a histidine in motif I and a serine in motif II is indicative of a nucleophilic mechanism for the PPIase reaction (28).

Rescue of Yeast Cell Death by the dodo (*dod*) Gene Product. To determine whether a transgenic dodo gene could restore viability to a yeast cell containing a mutated *ESS1* gene (denoted *ess1*), the dodo cDNA was placed under the control of a yeast constitutive TPI promoter. This construct (denoted pdodo) was introduced into diploid yeast cells in which both copies of *ESS1* were disrupted (*ess1/ess1*), but which are viable because they carry a plasmid-borne copy of the yeast gene (pESS1). We reasoned that if the (*ess1/ess1*; pESS1; pdodo) cells could be cured of pESS1 and still divide, then *ESS1* and dodo genes could be considered interchangeable in a functional sense.

It is seen that pESS1 is retained by cells when a vector without insert is introduced, but is lost ≈75% of the time when cells carry the pdodo cDNA in the "sense" orientation (Table 2). When the antisense dodo construct is introduced, the pESS1 plasmid is retained 100% of the time. Thus, the plasmid-borne dodo gene substitutes for the *ESS1* gene and allows growth of the *ess1/ess1* diploid cells.

Secondly, the dodo cDNA was introduced into a heterozygous yeast strain in which only one allele was disrupted and the other was normal (*ess1/ESS1*; pdodo). These cells were sporulated, and the resulting tetrads were dissected. As expected, cells transformed with the unloaded vector show a 2:2 segregation for viable:inviable spores (Table 3). In contrast to this, about half of the tetrads derived from (*ess1*/ESS1; pdodo) or (*ess1/ESS1*; pESS1) yield a 4:0 segregation of viable:inviable spores, indicating that the dodo cDNA in the "sense" orientation complements *ess1* haploid cells and allows spore outgrowth and cell viability. Cells transformed with the dodo antisense cDNA do not rescue the potential cell death of *ess1* haploid cells and yield a 2:2 viable:inviable segregation ratio.

Functional Interchangeability. Our finding that dodo and ESS1 proteins each contain a WW protein–protein binding domain raises the possibility of their involvement in a multisubunit complex, one in which protein–protein interactions might be influenced by proximity to the putative PPIase protein folding domain. Furthermore, the knowledge that PPIases occur in most cellular compartments; can form stable associations with transcription factors, receptors, and kinases; and aid in certain structural requirements for the activation of signaling molecules (26) suggests that the dodo protein may be involved in a number of different cellular processes. To

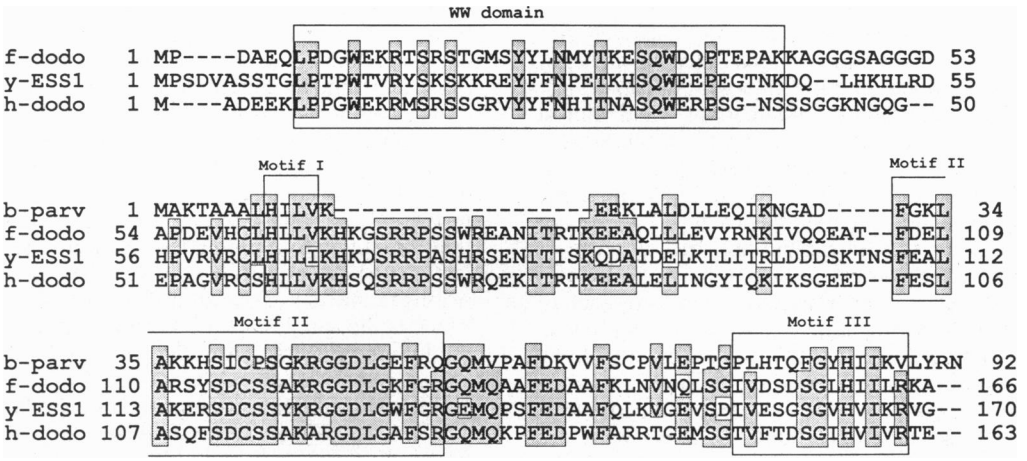

FIG. 2. Alignment of the predicted proteins of the bacterial parvulin (b-parv) (27), fly dodo (f-dodo), yeast ESS1 (y-ESS1) and human dodo (h-dodo). Identical residues in at least three proteins are shaded. The WW domain and the PPIase motifs are boxed. The yeast protein represents a corrected version of ESS1 (23, 24) and the human dodo has been assembled from overlapping EST clones from GenBank (accession numbers: H20422 and H18276).

determine this, we are now searching for its partners via the yeast two-hybrid system (S.D.H. and R.M., unpublished data). Furthermore, the comparative sequence information that we have drawn together on the WW domain has allowed us to perform site-directed mutagenesis to evaluate the critical residues involved in protein binding (S.D.H. and R.M., unpublished data).

The dodo gene–*ESS1* transgenic data add to the small number of fly genes that have been shown to substitute functionally for yeast genes. These include genes for adenine 8, topoisomerase II; CDC2; cyclin; ubiquitin-conjugating enzyme; the OCT2 transcription factor, an endoplasmic reticulum membrane-bound translocation protein and a serine-threonine phosphatase. By contrast, about 40 human genes have been shown to complement yeast mutants (31).

Genes in the flightless Region Appear Not To Be Members of Multigene Families. We sampled the fly genome by hybridization and extensive cDNA library screens at different developmental stages to determine whether tweety, flightless, dodo, or penguin have conserved relatives, whose products might compensate for the absence of each of these genes. All four transcription units appear as single bands on Southern analysis (unless they contain a repetitive element). More importantly, all of the cDNAs that we isolated arise as transcripts from each of the genes in question, and none come from other genomic regions. Thus, if active redundant copies of each of these genes do exist, they would have to be sufficiently diverged to be undetectable under our conditions of normal stringency. If highly diverged copies coding for similar proteins do indeed occur in the fly genome and compensate for any deficiency, their existence is only likely to be uncovered after the entire fly genome has been sequenced. However, even this is no guarantee, as is revealed by an example from vertebrates and plants. For example, the interleukin 1β and soybean trypsin inhibitor proteins are only recognizable as being nearly identical after elucidation of their three-dimensional crystallographic structures. They remain unrecognizable at both the DNA and amino acid sequence levels. Thus, we so far conclude that the tweety, dodo, and penguin genes are not part of conserved multigene families, and hence we consider the possibility, discussed below, that their absence is compensated for either by other unrelated proteins or by degenerate networks.

Redundancy and Degeneracy. As we have seen, the simultaneous deletion of three genes coding for three quite different proteins is compatible with development to adulthood for the majority of individuals. This high ratio of nonvital to vital genes is not a peculiarity of the flightless region *per se*, as an examination of the region adjacent to flightless reveals similar results (G.L.G.M., unpublished data). Of the seven transcription units proximal to the flightless region, five can be simultaneously deleted and some flies still complete development to adulthood. However, viability is low, and the survivors are uncoordinated and sluggish and rarely live for more than 2 days (G.L.G.M., unpublished data). Thus, of the 11 transcription units from tweety (*twe*) to *LB20*, only flightless (*fli*), *A112*, and *LB20* correspond to vital loci (Fig. 1). These data conform to the more general picture in *D. melanogaster*; a majority of loci are not vital under laboratory conditions.

The fact that function is relatively unperturbed after various deletions in the region under study requires a cogent explanation, and several possibilities suggest themselves. One, introduced earlier, is structural redundancy; the other is embedded in the combinatorial degeneracy hypothesis first proposed by Edelman in the context of nervous system function

Table 2. Probability of loss of the pESS1 in a diploid yeast disruption strain harboring constructs containing the dodo gene (*ess1/ess1*; pdodo, pESS1) in the absence of selection for retaining pESS1

Plasmid	His^+/Leu^+	Ura^+	% loss of pESS1
pvector	216/216	216	0
pdodo-1	42/216	216	81
pdodo-2	70/216	216	68
pdodo-anti	216/216	216	0

Plasmids pdodo-1 and -2 contain dodo in the sense orientation relative to the TPI promoter, whereas pdodo-anti contains dodo in the reverse orientation (genotype: *ess1*::*URA3*; pESS1, *HIS3*; and pdodo, *LEU2*).

Table 3. Tetrad analysis of an heterozygous *ess1/ESS1* yeast strain transformed either with pdodo or with pESS1 and induced to undergo sporulation

Plasmid	Tetrads dissected	Viable spores per tetrad				
		0	1	2	3	4
pvector	29	1	3	24	1	0
pdodo-1	30	0	3	13	2	12
pdodo-2	31	0	2	17	2	10
pdodo-anti	32	1	2	29	0	0
pESS1	29	0	0	6	6	17

Genotype: *ess1*::*URA3/ESS1*. In all cases ura^+ segregants were also leu^+ or his^+, indicating the presence of pdodo or pESS1, respectively.

(11, 12). Degenerate brain systems, for example, were defined as ones in which different neuronal groups carry out the same function, even though they share only some structural features in common; redundant systems, on the other hand, are ones where repeated units of identical structure carry out the same function (12). Thus, redundant systems are isofunctional and isomorphic, whereas degenerate systems are isofunctional but nonisomorphic. The molecular developmental aspects of this concept have been expanded in the context of cell adhesion, cell signaling, and morphogenesis (11).

Most authors have not distinguished between redundancy and degeneracy but have used redundancy in a generic way to include both. It is clear that the distinction is an important one and that in fact the opposite is true; redundancy is simply a special case of degeneracy (12).

Familiar structurally redundant systems in *D. melanogaster* are the large tandem arrays of histone and rRNA genes that are clearly isofunctional and isomorphic. More restricted arrays arising from local tandem duplications are found at the sloppy paired (*slp*), Bar (*B*), gooseberry (*gsb*), dopa decarboxylase (*Ddc*), engrailed (*en*), serendipity (*Sry*), cecropin (*Cec*), and Enhancer of split [*E(spl)*] loci. The extent to which each of the copies in a tandem duplication is isofunctional and isomorphic varies from locus to locus (32, 33).

The task of assigning a particular protein to degenerate, redundant, or lack-of-function categories is, in fact, enormously difficult. Certainly the claim by Capecchi that "if you give me a gene, I could knock it out and tell you what its function is" (34) is not borne out by the huge amount of molecular data from different eukaryotes that awaits placement into a functional biological context.

Although the current literature almost invariably opts for redundancy whenever the results of a gene knockout appear of minor phenotypic consequence to an investigator, at the present time the activities of degenerate systems provide an equally likely alternative. Degenerate systems are well known in signal transduction pathways where different ligand-receptor systems converge to carry out the same function (35); these are clearly isofunctional but nonisomorphic. At the metabolic level, there are a large number of alternative pathways which can produce the same functional product, an excellent example being the degeneracy involved in the conversion of lanosterol to ergosterol in *S. cerevisiae* (36).

To understand how developmental and evolutionary transitions occur in the presence of compensatory networks, studies of the same gene and its interacting partners are required in different phyla. In the case of the flightless and dodo proteins, which each contain different protein binding domains, we are now searching for the components of the protein–protein complexes in which they are engaged via the two-hybrid system in yeast. The interacting proteins can then be sought and isolated from other eukaryotes, and the degree of commonality in this small part of each network can be evaluated. This iterative cycling between phyla, together with transgenic evaluations between phyla, is fundamental to understanding the principles and evolutionary consequences of redundancy and degeneracy at different levels. Our continuing isolation and mapping of human, worm, and yeast homologs for different genes in this well-characterized region is one more step in this direction (21, 37).

We thank J. Cotsell, F. Hall, D. Hall, A. Kasprzak, and J. Mason for technical help and Fred Jones and Kathryn Crossin for their insights and suggestions on reorganization of the manuscript. S.D.H. and R.L.H. were supported by Health Research Inc. H.G.d.C. was supported by National Science Foundation Grant DCB-9106129 and by Australian Research Council Grant AO 8716002, and acknowledges the assistance of Ms. Ozsarac during the early sequencing phase of the dodo project. R.M. and G.L.G.M. were funded by the Australian National University and the Neurosciences Support Foundation. While this work was in progress we became aware that a human homolog of *ESS1* had been isolated in the Hunter Laboratory at the Salk Institute.

1. Friedrich, G. & Soriano, P. (1991) *Genes Dev.* **5,** 1513–1523.
2. Erickson, H. P. (1993) *J. Cell Biol.* **120,** 1079–1081.
3. Crossin, K. L. (1994) *Perspect. Dev. Neurobiol.* **2,** 21–32.
4. Soriano, P. (1995) *Annu. Rev. Neurosci.* **18,** 1–18.
5. Goebl, M. G. & Petes, T. D. (1986) *Cell* **46,** 983–992.
6. John, B. & Miklos, G. L. G. (1988) *The Eukaryote Genome in Development and Evolution* (Allen and Unwin, London).
7. Johnsen, R. C. & Baillie, D. L. (1991) *Genetics* **129,** 735–752.
8. Ferguson, E. L. & Horvitz, H. R. (1989) *Genetics* **123,** 109–212.
9. Brenner, S., Dove, W., Herskowitz, I. & Thomas, R. (1990) *Genetics* **126,** 479–486.
10. Tautz, D. (1992) *BioEssays* **14,** 263–266.
11. Edelman, G. M. (1993) *Cell Adhes. Commun.* **1,** 1–7.
12. Edelman, G. M. (1987) *Neural Darwinism* (Oxford Univ. Press, Oxford).
13. Miklos, G. L. G. (1993) *Mem. Assoc. Aust. Palaeontol.* **15,** 7–41.
14. Miklos, G. L. G. (1993) *J. Neurobiol.* **24,** 842–890.
15. Miklos, G. L. G., Campbell, K. S. W. & Kankel, D. R. (1994) in *Flexibility and Constraint in Behavioral Systems*, eds. Greenspan, R. J. & Kyriacou, C. P. (Wiley, New York), pp. 269–293.
16. Miklos, G. L. G. & Campbell, K. S. W. (1994) in *Early Life on Earth*, ed. Bengston, S. (Columbia Univ. Press, New York), pp. 501–516.
17. Perrimon, N., Smouse, D. & Miklos, G. L. G. (1989) *Genetics* **121,** 313–331.
18. Miklos, G. L. G. & de Couet, H. G. (1990) *J. Neurogenet.* **6,** 133–151.
19. Delaney, S. J., Hayward, D. C., Barleben, F., Fischbach, K.-F. & Miklos, G. L. G. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7214–7218.
20. Hayward, D. C., Delaney, S. J., Campbell, H. D., Ghysen, A., Benzer, S., Kasprzak, A. B., Cotsell, J. N., Young, I. G. & Miklos, G. L. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2979–2983.
21. Campbell, H. D., Schimansky, T., Claudianos, C., Ozsarac, N., Kasprzak, A. B., Cotsell, J. N., Young, I. G., de Couet, H. G. & Miklos, G. L. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11386–11390.
22. de Couet, H. G., Fong, K. S. K., Weeds, A. G., McLaughlin, P. J. & Miklos, G. L. G. (1995) *Genetics* **141,** 1049–1059.
23. Hanes, S. D., Shank, P. R. & Bostian, K. A. (1989) *Yeast* **5,** 55–72.
24. Hani, J., Stumpf, G. & Domdey, H. (1995) *FEBS Lett.* **365,** 198–202.
25. Schmid, F. X. (1993) *Annu. Rev. Biophys. Biomol. Struct.* **22,** 123–143.
26. Rutherford, S. L. & Zuker, C. S. (1994) *Cell* **79,** 1129–1132.
27. Rahfeld, J-U., Rücknagel, K. P., Schelbert, B., Ludwig, B., Hacker, J., Mann, I. & Fischer, G. (1994) *FEBS Lett.* **352,** 180–184.
28. Rudd, K. E., Sofia, H. J., Koonin, E. V., Plunkett, G., Lazar, S. & Rouviere, P. E. (1995) *Trends Biochem. Sci.* **20,** 12–14.
29. Sudol, M., Bork, P., Einbond, A., Kastury, K., Druck, T., Negrini, M., Huebner, K. & Lehman, D. (1995) *J. Biol. Chem.* **270,** 14733–14741.
30. Chen, H. I. & Sudol, M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7819–7823.
31. Tugendreich, S., Bassett, D. E., Jr., McKusick, V. A., Boguski, M. S. & Hieter, P. (1994) *Hum. Mol. Genet.* **3,** 1509–1517.
32. Cadigan, K. M., Grossniklaus, U. & Gehring, W. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6324–6328.
33. Maier, D., Marte, B. M., Schafer, W., Yu, Y. & Preiss, A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 5464–5468.
34. Travis, J. (1992) *Science* **256,** 1392–1394.
35. Geraerts, W. P. M., Smit, A. B. & Li, K. W. (1994) in *Flexibility and Constraints in Behavioral Systems*, eds. Greenspan, R. J. & Kyriacou, C. P. (Wiley, Chichester, U.K.), pp. 209–235.
36. Fryberg, M., Oehischlager, A. C. & Unrau, A. M. (1973) *J. Am. Chem. Soc.* **95,** 5747–5757.
37. Chen, K.-S., Gunaratne, P. H., Hoheisel, J. D., Young, I. G., Miklos, G. L. G., Greenberg, F., Shaffer, L. G., Campbell, H. D. & Lupski, J. R. (1995) *Am. J. Hum. Genet.* **56,** 175–182.